Prion protein deficiency impairs hematopoietic stem cells determination and sensitizes myeloid progenitors to irradiation

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Prion protein deficiency impairs hematopoietic stem cells determination and sensitizes myeloid progenitors to irradiation

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Running title: PrPC-dependent hematopoietic homeostasis radioprotection of hematopoietic progenitors.

Abstract

Highly conserved among species and expressed in various types of cells, numerous roles have been attributed to the cellular prion protein (PrPC). In hematopoiesis, PrPC regulates hematopoietic stem cells self-renewal but the mechanisms involved in this regulation are unknown. Here we show that PrPC regulates hematopoietic stem cells number during aging and their determination towards myeloid progenitors. Furthermore, PrPC protects myeloid progenitors against the cytotoxic effects of total body irradiation. This radioprotective effect was associated with increased cellular prion mRNA level and with stimulation of the DNA repair activity of the Apurinic/pyrimidinic endonuclease 1, a key enzyme of the base excision repair pathway. Altogether, these results show a previously unappreciated role of PrPC in adult hematopoiesis and indicate that PrPC mediated stimulation of BER activity might protect hematopoietic progenitors from the cytototic effects of total body irradiation.

Key words: prion protein; radiation; hematopoiesis; myeloid progenitors; base excision repair.

Introduction

Radiotherapy is commonly used alone or in combination with genotoxic drugs for treatment of numerous solid tumours. Despite progress in its targeting, radiotherapy can be deleterious to two tissues, the gastrointestinal tract and the bone marrow, and can lead to secondary effects commonly defined as the Acute Radiation Syndrome[1]. Irradiation of the bone marrow damages hematopoietic stem and progenitor cells (HSPC) and perturbs the hematopoietic microenvironment[2,3] resulting in radiation-induced acute myelosuppression[4,5] and increased susceptibility to infections[6,7].

Numerous types of DNA lesions are induced by ionizing radiation exposure of cells. They include base modifications, apurinic/apyrimidic sites (AP sites), single- (SSB) and double (DSB)-strand breaks. DSBs are the main lesions affecting cell survival. They can arise not only directly by energy deposition on the DNA but also as a consequence of the formation of AP sites or SSBs[8,9]. Indeed, base excision repair (BER) activities, and in particular the processing of abasic sites, have been shown to contribute to radiation-induced DNA damage[10,11].

AP endonuclease-1 (Ape1) is the unique enzyme that converts AP sites into single strand break intermediates during BER. Ape1 3'-phosphodieterase and –phosphate activities (for review [12]) also contribute to the processing of radiation-induced DNA strand break extremities[13] during the single strand break repair pathway (SSBR). Accordingly, protection of neuronal cells from radiation-induced damage requires Ape1[14,15].

The Prion protein (PrPC) is a highly conserved glycoprotein that, when structurally modified, plays a critical role in the pathogenesis of neurodegenerative disorders called prion diseases[16]. The normal Prion protein was shown to protect cells from oxidative stress[17–20]. It also protects from DNA damage by promoting APE1 DNA repair activity and cell survival through an interaction with APE1[21]. During hematopoiesis, PrPC is highly expressed in HSC and hematopoietic progenitors[22–24] and PrPC deficiency is associated with decreased HSC self-renewa[25]l. As oxidative stress and DNA damage act on HSC cell fate[26], PrPC might participate in the maintenance of the hematopoietic system and its response to cytotoxic stresses. To address these points, we used *Prnp* knockout mice to study the consequences of

PrPC deficiency on hematopoiesis of young and old adult mice and on the response of HSC and hematopoietic progenitors to gamma-irradiation.

Methods:

Mice

Mice experiments were carried out in compliance with the European Community Council Directive (EC/2010/63) and were approved by our Institutional ethics committee (CEtEA-CEA-DRF–N°17-096). The B6.129S7-Prnptm1Cwe/Orl mice were from the European Mutant Mouse Archive and bred in our animal facility. We also used Prnp $^{ZH3/ZH3}$ mice provided by A. Aguzzi (Zurich, Switzerland) and C57BL/6 mice were purchased from Charles River.

Cell sorting and flow cytometry analysis of bone marrow cells.

Murine bone marrow cells were flushed out of femurs, tibiae, hipbone and humeruses using a syringe filled with DPBS and filtered through a 70 µm-cell strainer. After red blood cell lysis using NH_4Cl solution (STEMCELL Technologies), mononuclear cells were phenotyped using different antibody cocktails from Biolegend, e-Bioscience or Beckton Dickinson. Flow cytometry analysis was performed with a BD FACSLSRII^{TM} flow cytometer (BD Biosciences) and cell sorting with a FACS Influx cell sorter (Becton Dickinson). Data were analyzed with FlowJo software. Antibodies and gating strategies for hematopoietic subset analysis and sorting are described in supplementary methods. For RT-PCR and Ape1 endonuclease activity experiments, aliquots of 50 000 cells myeloid progenitors were sorted in PBS whereas aliquots of 10 000 HSC and MPP were sorted in PBS/1%BSA.

Ape1 endonuclease activity

Cell extracts were obtained by sonication of pelleted bone marrow sorted cells in 20mM Tris-HCl, pH 7.5, 250mM NaCl, 1mM EDTA, 20mM sucrose, and protease inhibitor cocktail 0.1% (Sigma-Aldrich P2714). For progenitor analysis, 50 000 cells were suspended in 125µl extraction buffer. For HSC or MEP analysis, 10 000 sorted cells were suspended in 30µl extraction buffer. After sonication, the homogenate was centrifuged at 20 000 x g for 30 min at 4°C and aliquots of the supernatant were stored at -80°C. Ape1 endonuclease activity was measured using a 5'-end labelled 34-mer oligonucleotide containing a single tetrahydrofuranyl artificial AP site at position 16 hybridized to its complementary oligonucleotide containing a cytosine opposite the lesion (Eurogentec). For measuring Ape1 activity in bone marrow

progenitors, 1 to 4µl of cell extract were incubated for 30min at 30°C in 10µl of reaction buffer containing 25mM Tris-HCl pH 7.5, 5mM $MgCl_2$, 5% glycerol, 52mM NaCl, BSA 1µg/µl and 150 fmoles of the hybridized oligonucleotide. For determining Ape1 activity in bone marrow HSC and MPP, 1 to 4µl of cell extract were incubated for 10min at 30°C in 10µl reaction buffer containing 25mM Tris-HCl pH 7.5, 5mM $MgCl_2$, 5% glycerol, 52mM NaCl, and 150 fmoles of the hybridized oligonucleotide. The reaction was stopped by adding 4µl of denaturating buffer (80 % formamide, 0.1% bromophenol blue, 10mM EDTA) followed by heating for 5 min at 95°C. The products of the reaction were resolved by denaturating 7M urea-20% polyacrylamide gel electrophoresis. Gels were scanned using a Typhoon 5 (GE Healthcare Life Sciences) and band intensifies were quantified with ImageQuant TL 8.1 (GE Healthcare Life Sciences).

Statistical analyses

Quantitative data are presented as the mean ± sem. Statistical significance was assayed using the non-parametric Mann Whitney U-test (GraphPad Prism software).

Additionnal material and methods are found in Supplementary Methods.

Results:

PrPC is differentially expressed in hematopoietic stem cells and myeloid progenitors and is involved in HSC expansion during aging

To address the potential functions of PrPC in hematopoietic stem and progenitor cells (HSPC), we first characterized the expression pattern of *Prnp* in different purified hematopoietic sub-subpopulations i.e. common Myeloid Progenitors (CMP), Granulocyte-Monocyte Progenitors (GMP), Megakaryocyte-Erythrocyte Progenitors (MEP), multi-potent progenitors (MPP) and hematopoietic stem cells (HSC). The highest level of Prnp mRNA was found in MEP while they were 2.7-fold and 4.3-fold lower in CMP and GMP, respectively (Fig 1A). These differences in mRNA expression were also found at the protein level (Fig 1B and Fig S1A). The *Prnp* mRNA level in purified HSC was 2.5 fold higher than in MPP (Fig 1C).

To determine if PrPC has a role in hematopoiesis, we first compared bone marrow (BM) from 3 months-old $Prnp^{+/+}$ (WT) and $Prnp^{-/-}$ (KO) mice. WT and KO mice showed similar peripheral blood counts (data not shown) and BM cellularity (Fig S1B). However, the frequency of CMP, GMP and MEP was significantly reduced in KO mice compared to WT mice (Fig 1D), whereas CLP, MPP, ST- and LT-HSC frequencies were similar (Fig 1E). The differences between KO and WT myeloid progenitor frequencies were not associated with increased apoptosis (Fig S1C) or cell cycle alteration (Fig S1D) but with a higher percentage of quiescent MPP and ST-HSC (Fig 1F). These results suggest a defect of determination of HSPC (MPP and ST-HSC) towards the myeloid lineage in $Prnp^{-/-}$ mice. Finally, clonogenic assay using purified CMP and GMP showed a significantly decreased plating efficiency of $Prnp^{-/-}$ CMP and GMP (Fig 1G). Taken together, these results show intrinsic myeloid differentiation deficiencies in $Prnp^{-/-}$ HSC and progenitors and suggest that the reduction of cycling MPP and ST-HSC contributes to the lower myeloid progenitor content in the BM from KO compared to WT mice.

Aging of HSC is associated with increased in the percentage of HSC in BM, decreased HSC self-renewal and accumulation of DNA damage[27]. As PrPC has been implicated in HSC self-renewal[25] and in DNA repair[21], we investigated the effect of PrPC loss in HSC numbers and DNA repair capacity during aging in $Prnp^{+/+}$ and $Prnp^{-/-}$ mice. *Prnp* mRNA level in HSC was 2.7-fold higher in 11 months-old

compared to 3 months-old mice (Fig 1H) but did not change in MPP (Fig 1H). BM from 11 months-old WT and KO mice displayed similar cellularity (Fig S1E). As in 3 months-old mice, a lower frequency of myeloid progenitors but not MPP (Fig 1I) was found in 11 months-old KO mice (Fig S1F). In contrast, compared to their 3 months-old counterparts, ST- and LT-HSC frequencies respectively increased 2- and 8.3-fold in 11 months-old WT mice but did not change (ST-HSC) or was only 3-fold increased (LT-HSC) in 11 months-old KO mice (Fig 1I). DNA repair was slightly dependent on PrPC in aged HSC. A 1.4 fold increased activity of Ape1 in WT HSC was found between 3 months and 11 months without any change in Ape1 mRNA level. In KO HSC, Ape1 activity also increased between 3 months and 11 months, but to a lesser extent (1.2-fold) than in WT HSC. Interestingly, this increased activity was associated with an increased Ape1 mRNA level (Fig 1J and Fig 1K).

Altogether, these results show that PrPC deficiency is associated with decreased HSC determination towards the myeloid lineage and decreased number of HSC and decreased APE1 activity in old mice.

Prnp expression is upregulated in myeloid progenitors and HSC subpopulations after in vivo radiation exposure

HSC aging is associated with increased oxidative stress[28] and PrPC has been shown to protect cells from oxidative stress[17–20]. To characterize PrPC role during an oxidative stress of hematopoiesis, we performed total body irradiation (TBI) on WT and KO mice. Survival curves of WT and KO mice exposed to increasing gamma-radiation doses showed that a higher percentage of irradiated KO mice died earlier than irradiated WT mice even if statistical significance between both genotypes was not reached ($p=0.0792$ at 7 Gy) (Fig 2A). When KO mice were grafted with BM from non-irradiated WT mice 24h after a 7 Gy irradiation, they did not die, indicating that the higher sensitivity of KO mice to TBI was not due to the bone marrow microenvironment of KO mice and that they died from hematopoietic syndrome.

One hour after a 7 Gy TBI, a 1.5-fold increase of *Prnp* mRNA level was found in HSC, CMP and GMP but not in MPP and MEP (Fig 2B). These data are consistent with the observed *Prnp* upregulation in neuronal tissues after exposure to genotoxic stress[21] and suggest a potential role of PrPC in response to radiation in GMP, CMP, and HSC.

PrPC-dependent increase in the DNA repair activity of Ape1 is associated with radioprotection of CMP and GMP

PrPC prevents cell death in response to alkylating agent or H_2O_2 exposure by directly stimulating the DNA repair activity of Ape1[21]. Without irradiation, Ape1 activity was similar in WT and KO progenitors (Fig 2C). One hour after a 7 Gy TBI, Ape1 activity increased in all WT irradiated myeloid progenitors analyzed (from 1.5 to 1.7 fold) but not in their KO counter parts (Fig 2D). In HSC and MPP Ape1 activity was similar in WT and KO mice (Fig 2E) but increased only in WT HSC after a 7 Gy TBI (Fig 2F). Whatever the subpopulation analyzed, the radiation-induced Ape1 activity was not associated with an increase in Ape1 mRNA level (Fig S2A and S2B). These data show that after irradiation, Ape1 activity in all myeloid progenitor subpopulations and in HSC is stimulated in a PrPC-dependent manner.

As the radiation-induced death of myeloid progenitors is dependent on apoptosis[5], we quantified apoptosis in WT and KO myeloid progenitors one and twelve hours after TBI at 7 Gy. One hour after irradiation, apoptotic (Annexin V-positive cells) and dead cells (Annexin V-negative and Hoechst-positive cells) fractions increased only in CMP and were higher in KO compared to WT CMP (Fig 2G and S2C). Twelve hours after irradiation, both apoptotic (Fig 2H) and dead (Fig S2D) cells fractions increased in all myeloid progenitor subpopulations in both WT and KO mice. However, higher rates of apoptosis and cellular death were observed in irradiated KO compared to WT GMP. In accordance, significantly lower frequencies of CMP and GMP were found in KO versus WT irradiated mice 18h after irradiation (Fig 2I). Myeloid progenitors from *Prnp* $^{ZH3/ZH3}$ mice exhibited the same radiation sensitivity than those from $Prnp^{-/-}$ mice shown by a similar reduced number compared to mice in non-irradiated conditions (Fig S2 E). Altogether these results suggest that PrPC-dependent stimulation of the DNA repair activity of Ape1 is required for the radioprotection of myeloid progenitors.

Discussion

Despite numerous studies, the physiological role of PrPC remains elusive. Recently, we showed that PrPC can stimulate an important DNA repair pathway, the BER, in neuronal tissues through interaction with and stimulation of its key enzyme, APE1[21]. Here we show that the same mechanism can be proposed for the radioprotection of myeloid progenitors, HSC determination, and the expansion of the HSC compartment during aging.

Previous studies[22–24,29] indicated a decreased *Prnp* expression during differentiation of hematopoietic cells. Here, we performed an extended study of *Prnp* expression in different hematopoietic sub-populations and showed a 3-fold higher *Prnp* expression level in MEPs compared to their progenitors CMP suggesting that the correlation between *Prnp* downregulation and cellular differentiation[24] may not be a general feature in hematopoiesis. Furthermore, and contrary to a previous study[25], we found that KO mice have less myeloid progenitors. This discrepancy could be explained by the fact that in the previous study younger mice were analyzed (7 to 10 weeks-old mice compared to 3 months- and 11 months-old mice used in the present study) and by the number of backcrosses (4 versus >10) that might influence the phenotype of *Prnp* knockout mice[30]. Finally, we found a higher frequency of KO ST-HSC and KO MPP in the G0 phase. These populations being the direct precursors of myeloid progenitors, the increased quiescence of these cells might account for the decreased myeloid progenitor subpopulations[31]. Strinkingly, both KO CMP and GMP exhibited a lower plating efficiency despite no significant change in their cell cycle *in vivo*. Whether the microenvironment of these cells could compensate *in vivo* for an intrinsic growth deficiency observed *in vitro* remains to be clarified.

Prnp expression in the HSC compartment increased 8-fold with age. This higher expression was associated with the known elevated frequency of both ST- and LT-HSC[27,32]. PrpC deficiency was associated with no increase of ST-HSC and with a diminished increase of LT-HSC with age suggesting that PrPC plays a role in the age-dependent increase of HSC. Although aging-associated increase in HSC numbers has been known for a long time and is known to be cell-intrinsic[33,34], the underlying mechanisms are not fully understood. The results presented here indicate a significant involvement of PrPC in the age-dependent increase of HSC frequency. This increase may be accounted for by a PrPC-dependent up-regulation of Ape1

repair activity. Independent from its DNA repair activity[35,36]. Ape1 has a redox activity shown to be necessary for normal embryonic hematopoiesis[37], stem cell pool maintenance[38,39] and hematopoietic progenitors colony formation[40]. Thus, the PrPC-dependent stimulation of the APE1 DNA repair activity might contribute to hematopoietic homeostasis.

We found a modest but recurrent radiation sensitization of *Prnp* KO mice that contrasts with a previous study showing that the absence of PrPC protected rather than sensitized mice to an 8 Gy TBI[41]. However, this work was performed on a mixed 129/C57BL6 background, whereas we used a pure C57BL6 background. Furthermore, that study used a dose of X-rays that was lethal for WT animals while we used different doses of non-lethal γ-rays. Bone marrow myeloid cells are particularly sensitive to chemical and radiation cytotoxicity[4,42,43]. Accordingly, we found a dramatic decrease in bone marrow myeloid progenitors within the first 24h after radiation exposure that was exacerbated in *Prnp* KO irradiated mice. The reduced frequency of KO irradiated myeloid progenitors was associated with higher CMP and GMP apoptosis, within the first twelve hours after irradiation, as well as with an absence of stimulation of APE1 activity in these subpopulations one hour after irradiation. In contrast, in WT irradiated myeloid progenitors and HSC, upregulation of *Prnp* gene expression was associated with an increase in Ape1 activity in these sub-populations. PrPC has been shown to protect HSC from myelotoxic injury by 5-FU, commonly used in chemotherapy[25] and we now extend its myeloprotective role to radiotherapy.

Finally, a similar basal reduced number of myeloid progenitors and a similar radiation sensitivity of myeloid progenitors were found in the co-isogenic *Prnp* $^{ZH3/ZH3}$ mouse line[44] and the *Prnp*$^{-/-}$ mouse line. These results rule out the involvement of any *Prnp* flanking genes polymorphism previously described by Nuvolone et al[45].

Altogether, these results suggest that PrPC is involved in the homeostasis of steady-state hematopoiesis and that PrPC dependent activation of base excision repair contributes to the radioprotection of the myeloid progenitors of the mouse bone marrow.

Author contributions: CS performed experiments and analyzed data. ND performed cell sorting. VB performed bone marrow grafting. NG performed experiments, analyzed data, and reviewed the manuscript. PH R and JP R reviewed the manuscript. AA provided *Prnp* $^{ZH3/ZH3}$ mice. JBS and AB designed and performed experiments, analyzed data, and wrote the manuscript.

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Figure legends:

Fig1: PrPC contributes to mouse hematopoietic homeostasis.

(A) qRT-PCR analysis of *Prnp* expression, normalized to *Rplp0* in the indicated bone marrow sub-populations: CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythrocyte progenitor purified by flow cytometry from BM of 3 months-old mice (n=7-9). Data are presented as mean±SEM. Means with different letters are significantly different ($P<0.05$).

(B) Flow cytometry analysis of the PrPC protein expression in the indicated bone marrow supopulations. The graph depicts ratio of median fluorescence intensity (MFI) in WT and KO control cells (n=5-6). Data are presented as mean±SEM. Means with different letters are significantly different ($P<0.05$) (CMP versus MEP p=0.06).

(C) qRT-PCR analysis of *Prnp* expression, normalized to *Actb* in HSC, hematopoietic stem cell (LSK $CD135^-$) and in MPP, multipotent progenitor (LSK $CD135^+$) purified by flow cytometry from BM of 3 months-old mice (n=9); Data are presented as mean±SEM. Statistically significant difference between HSC and MPP is indicated by asterisks (*** $P<0.001$).

(D) Frequencies of myeloid progenitors in WT (black bars) and KO (white bars) bone marrow from 3 months-old mice (n=6-10). Data are presented as mean±SEM. Statistically significant differences between WT and KO are indicated by asterisks (* $P<0.05$; ** $P<0.01$).

(E) Frequencies of lymphoid progenitors (CLP), MPP, ST- and LT-HSC in the bone marrow from WT (black bars) and KO (white bars) mice (n=6). Data are presented as mean±SEM.

(F) Distribution of WT (black bars) and KO (white bars) HSC and MPP in each phase of the cell cycle. Data are presented as mean±SEM. Statistically significant differences between WT and KO are indicated by asterisks (* $P< 0.05$; ** $P<0.01$).

(G) In vitro plating efficiency of CMP and GMP purified by flow cytometry from BM of WT (black bars) and KO (white bars) mice (n=6-9). Data are presented as mean±SEM. Statistically significant differences between WT and KO are indicated by asterisks (** $P<0.01$).

(H) qRT-PCR analysis of *Prnp* expression, normalized to *Actb* in WT and KO HSC and MPP purified by flow cytometry from bone marrow of 3 months and 11 months

old mice (n=6-9). Data are presented as the mean±SEM. Statistically significant differences between 3 and 11 months-old mice and between HSC and MMP subpopulations are indicated by hash signs and asterisks, respectively (*** or ### *P*<0.001)

(I) Frequencies of MPP, ST- and LT-HSC in BM from 3 months and 11 months old WT (black bars) and KO (white bars) mice. Data are presented as the mean±SEM fold change of the frequencies in 11 months relative to 3 months-old mice. Statistically significant differences according to age or genotype are indicated by asterisks and hash signs, respectively (n=6-10; ** or ## *P*<0.01).

J) Ape1 endonuclease activity in 3 (opened) and 11 (hatched) months-old WT (dark) and KO (light) HSC. Data are presented as the mean±SEM. Statistically significant differences according to age or genotype are indicated by asterisks and hash signs, respectively (n=4-5; * or # *P*<0.05).

K) qRT-PCR analysis of *Ape1* expression, normalized to *Actb* in WT (dark) and KO (light) HSC purified by flow cytometry from bone marrow of 3 (opened bars) and 11 (hatched bars) months-old mice. Data are presented as the mean±SEM. Statistically significant differences according to age or genotype are indicated by asterisks and hash signs, respectively (n=7-9; * or # *P*<0.05).

Fig 2: PrPC favours survival of mice exposed to moderate doses of γ-rays and protects CMP from radiation-induced death.

(A) Kaplan-Meier survival plots of WT (solid lines) and KO (dashed lines) mouse overall survival after whole-body irradiation at indicated doses (n= 5 (10 Gy); n=13 (6.5 Gy); n= 28 (7 Gy) for each genotype). The arrow points to the 100% survival of both KO and WT irradiated mice (7 Gy) after transplantation of WT bone marrow cells (n=5).

(B) qRT-PCR analysis of *Prnp* expression in HSC, MPP and myeloid progenitors one hour after irradiation (7 Gy) (n=6). *Prnp* RNA levels were normalized to *Actb* (HSC and MPP) or *Rplp0* (myeloid progenitors). Data are presented as the mean±SEM fold change of normalized *Prnp* RNA levels in irradiated relative to control cells. Statistically significant differences between irradiated and control are indicated by asterisks (* *P*<0.05; ** *P*<0.01).

(C) Ape1 endonuclease activity in myeloid progenitor subpopulations from WT (black bars) and KO (white bars) mice (n=5-8). Data are presented as the mean±SEM. Means with different letters are significantly different (P<0.05).

(D) Ape1 endonuclease activity in myeloid progenitors from WT (black bars) and KO (white bars) mice (n=5-8) one hour after irradiation (7 Gy). Data are presented as the mean±SEM fold change of Ape1 endonuclease in irradiated relative to non-irradiated control cells. Statistically significant differences between non-irradiated and irradiated cells are indicated by asterisks (* P<0.05; ** P<0.01).

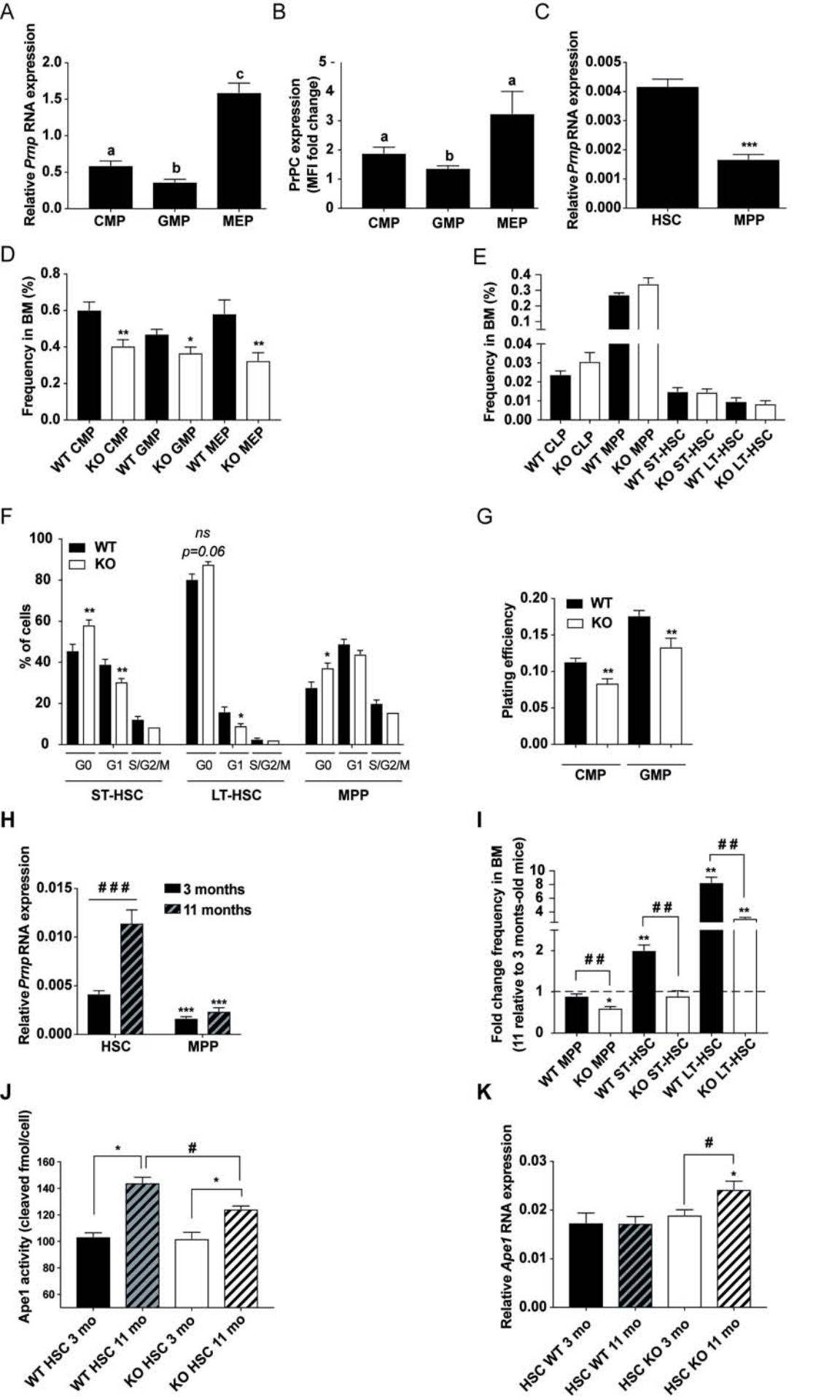
(E) Ape1 endonuclease activity in HSC and MPP from WT (black bars) and KO (white bars) mice (n=5-8). Data are presented as the mean±SEM.

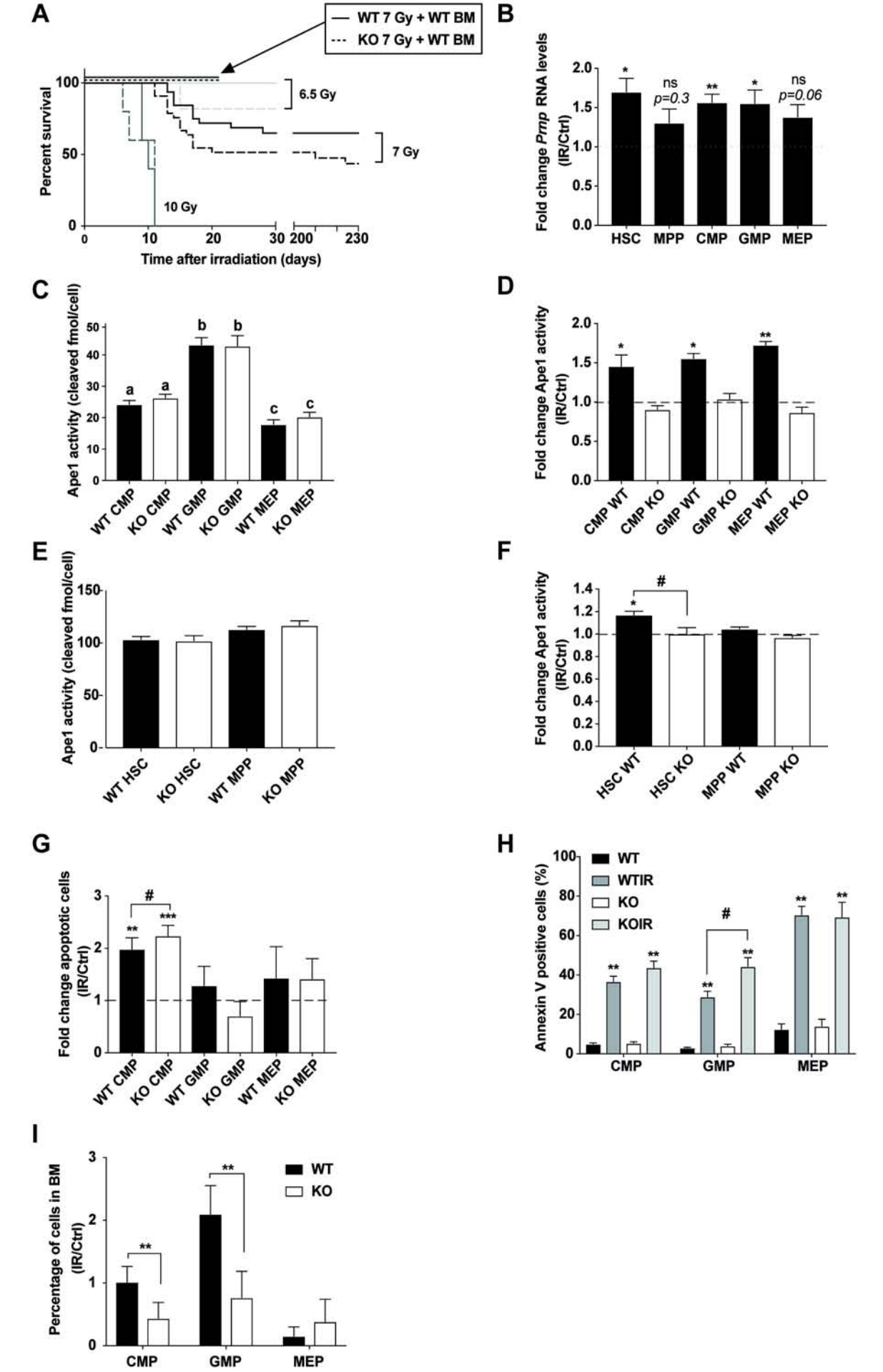
(F) Ape1 endonuclease activity in HSC and MPP from WT (black bars) and KO (white bars) mice (n=4-5) one hour after irradiation (7 Gy). Data are presented as the mean±SEM fold change of Ape1 endonuclease in irradiated relative to non-irradiated control cells. Statistically significant differences according to treatment or genotype are indicated by an asterisk and hash sign, respectively (* or # P<0.05).

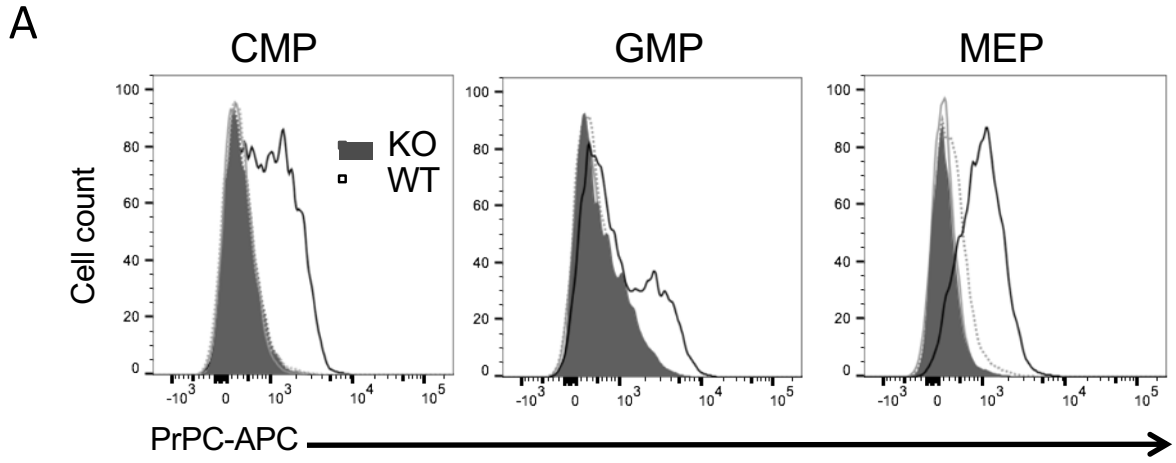
(G) Percentage of apoptotic myeloid progenitors (AnnexineV-positive cells) in BM from WT (black bars) and KO (white bars) mice (n=6-8) 1h after irradiation (7 Gy). Data are presented as the mean±SEM fold change of percentage of apoptotic cells in irradiated relative to non-irradiated control myeloid progenitors. Statistically significant differences according to treatment or genotype are indicated by asterisks (*) and hash signs, respectively (** P<0.01; *** P<0.001; # P<0.05).

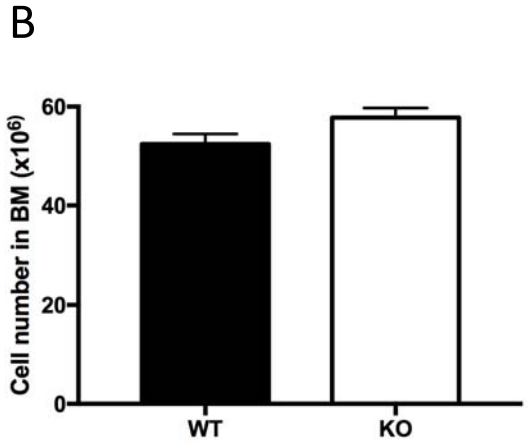
(H) Percentage of apoptotic myeloid progenitors (AnnexineV-positive cells) in BM from WT (dark gray bars - WTIR) and KO (light gray bars - KOIR) mice (n=6-8) 1h after irradiation (7 Gy). Data are presented as the mean±SEM. Non-irradiated control WT (black bars) and KO (white bars) myeloid progenitors are shown. Statistically significant differences according to treatment or genotype are indicated by asterisks (*) and hash signs, respectively (** P<0.01; # P<0.05).

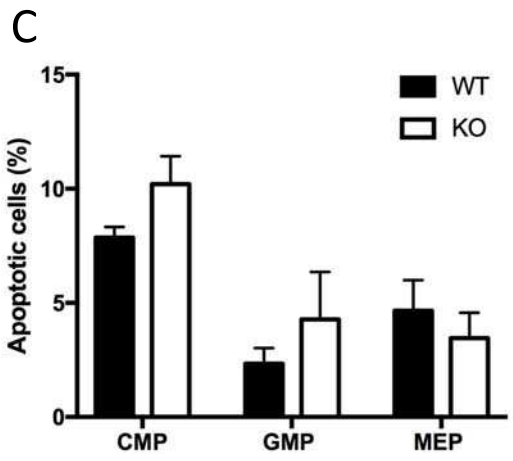
(I) Percentage of myeloid progenitors in BM from WT (black bars) and KO (white bars) mice (n=6-7) 18h after irradiation (7 Gy). Data are presented as the mean±SEM of the percentage of cells remaining in BM 18h after irradiation compared to non-irradiated control. Statistically significant differences between WT and KO mice are indicated by asterisks (** P<0.01).

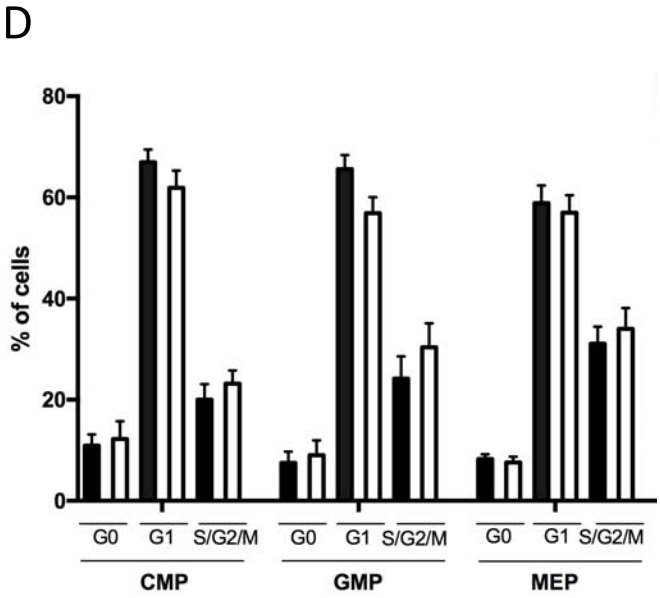

A
Relative Prnp RNA expression
2.0
1.5
1.0
0.5
0.0
a
b
c
CMP
GMP
MEP
B
PrPC expression (MFI fold change)
5
4
3
2
1
0
a
b
a
CMP
GMP
MEP
C
Relative Prnp RNA expression
0.005
0.004
0.003
0.002
0.001
0.000
***
HSC
MPP
D
Frequency in BM (%)
0.8
0.6
0.4
0.2
0.0
**
*
**
WT CMP
KO CMP
WT GMP
KO GMP
WT MEP
KO MEP
E
Frequency in BM (%)
0.4
0.3
0.2
0.1
0.04
0.03
0.02
0.01
0.00
WT CLP
KO CLP
WT MPP
KO MPP
WT ST-HSC
KO ST-HSC
WT LT-HSC
KO LT-HSC
F
100
80
60
40
20
0
% of cells
WT
KO
ns
p=0.06
**
**
*
*
G0
G1
S/G2/M
ST-HSC
LT-HSC
MPP
G
Plating efficiency
0.20
0.15
0.10
0.05
0.00
WT
KO
**
**
CMP
GMP
H
Relative Prnp RNA expression
0.015
0.010
0.005
0.000
###
3 months
11 months
***
***
HSC
MPP
I
Fold change frequency in BM (11 relative to 3 monts-old mice)
10
8
6
4
2
1
0
##
##
##
**
**
**
*
WT MPP
KO MPP
WT ST-HSC
KO ST-HSC
WT LT-HSC
KO LT-HSC
J
Ape1 activity (cleaved fmol/cell)
160
140
120
100
80
60
*
#
*
WT HSC 3 mo
WT HSC 11 mo
KO HSC 3 mo
KO HSC 11 mo
K
Relative Ape1 RNA expression
0.03
0.02
0.01
0.00
#
*
HSC WT 3 mo
HSC WT 11 mo
HSC KO 3 mo
HSC KO 11 mo

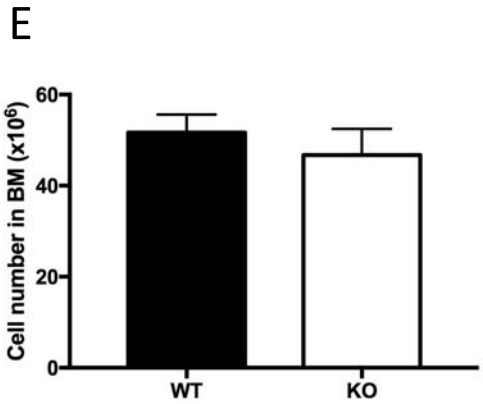

A
WT 7 Gy + WT BM
KO 7 Gy + WT BM
Percent survival
100
50
0
6.5 Gy
7 Gy
10 Gy
0
10
20
30
200
230
Time after irradiation (days)
B
Fold change Prnp RNA levels (IR/Ctrl)
2.0
1.5
1.0
0.5
0.0
*
ns
p=0.3
**
*
ns
p=0.06
HSC
MPP
CMP
GMP
MEP
C
Ape1 activity (cleaved fmol/cell)
50
40
30
20
10
0
a
a
b
b
c
c
WT CMP
KO CMP
WT GMP
KO GMP
WT MEP
KO MEP
D
Fold change Ape1 activity (IR/Ctrl)
2.0
1.5
1.0
0.5
0.0
*
*
**
CMP WT
CMP KO
GMP WT
GMP KO
MEP WT
MEP KO
E
Ape1 activity (cleaved fmol/cell)
150
100
50
0
WT HSC
KO HSC
WT MPP
KO MPP
F
Fold change Ape1 activity (IR/Ctrl)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
#
*
HSC WT
HSC KO
MPP WT
MPP KO
G
Fold change apoptotic cells (IR/Ctrl)
3
2
1
0
#
**
***
WT CMP
KO CMP
WT GMP
KO GMP
WT MEP
KO MEP
H
Annexin V positive cells (%)
100
80
60
40
20
0
WT
WTIR
KO
KOIR
**
#
CMP
GMP
MEP
I
Percentage of cells in BM (IR/Ctrl)
3
2
1
0
WT
KO
**
**
CMP
GMP
MEP

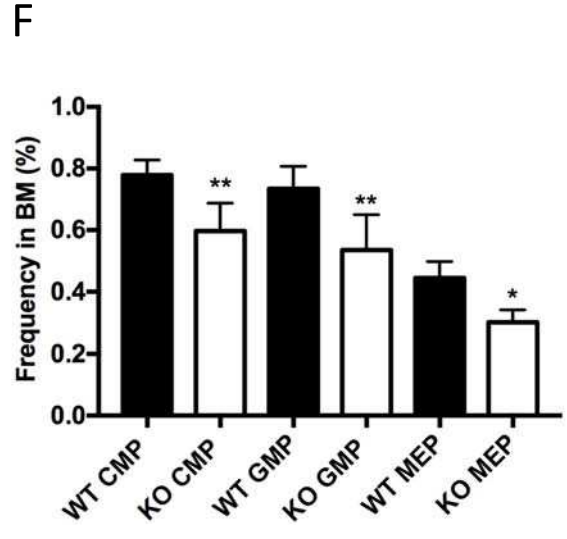

A
CMP
GMP
MEP
KO
WT
Cell count
PrPC-APC

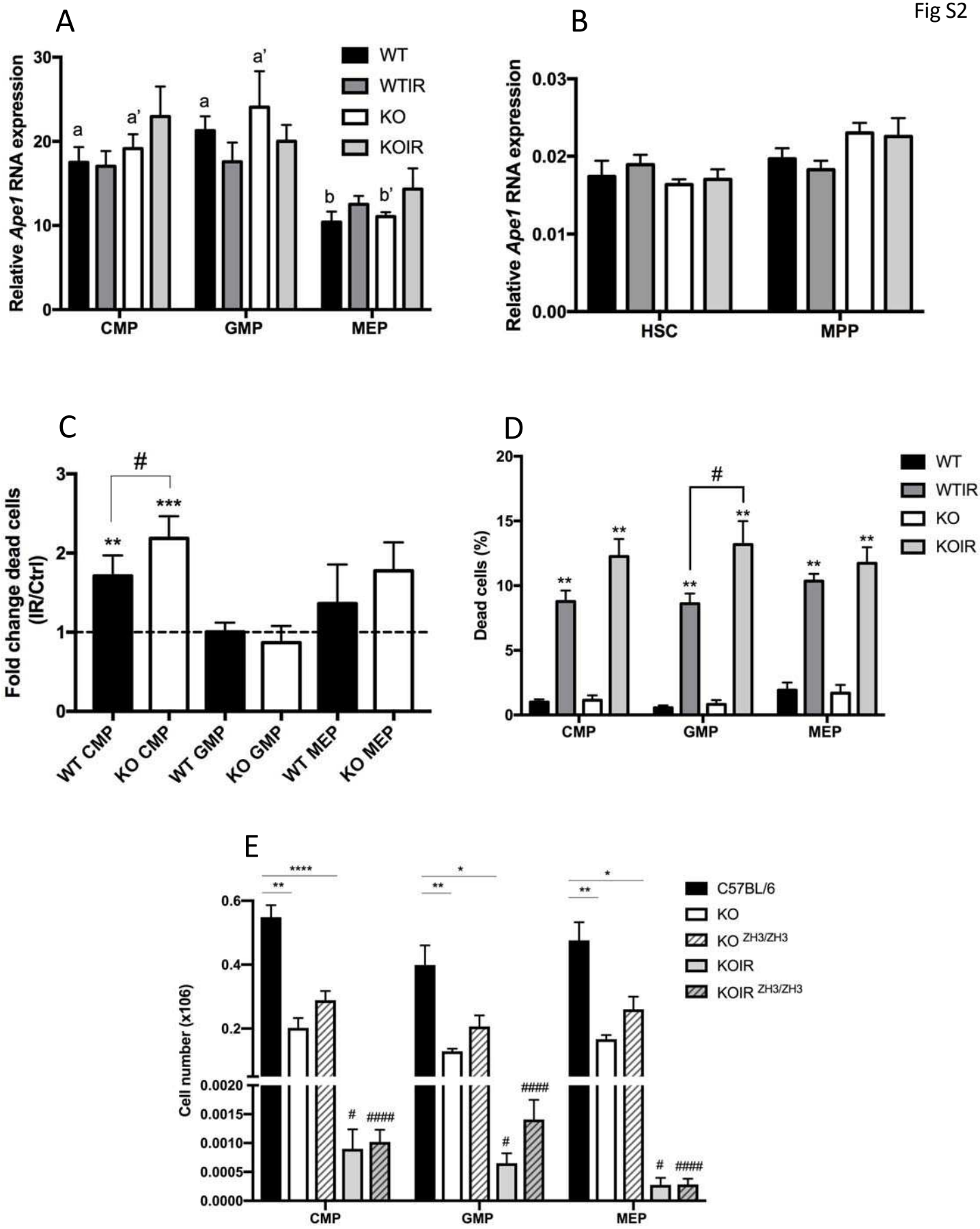

B
Cell number in BM (x10^6)
WT
KO


C
Apoptotic cells (%)
WT
KO
CMP
GMP
MEP


D
% of cells
G0
G1
S/G2/M
CMP
GMP
MEP


E
Cell number in BM (x10^6)
WT
KO


F
Frequency in BM (%)
WT CMP
KO CMP
WT GMP
KO GMP
WT MEP
KO MEP


A
Relative Ape1 RNA expression
WT
WTIR
KO
KOIR
a
a'
a
a'
b
b'
CMP
GMP
MEP
B
Relative Ape1 RNA expression
HSC
MPP
C
Fold change dead cells (IR/Ctrl)
#
**
***
WT CMP
KO CMP
WT GMP
KO GMP
WT MEP
KO MEP
D
Dead cells (%)
WT
WTIR
KO
KOIR
#
**
CMP
GMP
MEP
E
Cell number (x106)
C57BL/6
KO
KO ZH3/ZH3
KOIR
KOIR ZH3/ZH3
****
**
*
#
####
CMP
GMP
MEP